A language for modeling the morphogenesis process of biological system using MAS

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Abstract. The biological systems are Complex Adaptive Systems that can build a complex structure using interaction between cell and its environment. The process is called morphogenesis, it is study by developmental biology and applied by tissue engineering and regenerative medicine. Computational models are used by researchers to organize and communicate ideas, test hypotheses and abstractly represent behaviors, etc. Although, Agent Based Models are used in biological systems, it is necessary appropriate domain-oriented language. This work shows the progress made in the definition of a language for modeling and simulation of such systems, in order to hide details of implementation and execution of a simulation. The language concepts are demonstrated using, as study case, the morphogenesis of a heart valve in vitro.

1 Introduction

The developmental biology, is a biology' branch that study all biological process which cause that a developing organism arrives an ending form. This process is called morphogenesis, it is applied in regenerative medicine and tissue engineering to develop replacements of injured organs or tissues. There are discrete models where different authors trying to explain the phenomenon considered the cells dynamic and behavior. Perhaps, the most used is Glazier, Graner y Hogewek (GGH) model, aka Cellular Pots Model [2,3]. It apply a variant of Montecarlo method based in the Adhesion differential Hypothesis. In last years, different authors have done biological models applying ABM: the epithelial tissue morphogenesis in vitro, was studied by Grant et al [4]. Also, the process that control the epidermal homeostasis was researched by Schaller et al [11]. A tridimensional model of human epidermis to study the influence of growth factor (in biology it is equivalent to substance) TGF-β1 (Transforming Growth Factor Beta 1) in wound healing was made by Adra et al [1]. Except Adra et al, agent based models have been applied considering only geometrical aspects, like number of

neighbors or distance to a cluster of stems cells (niche), to simulate the cellular process of reproduction or differentiation.. However, Adra et Al, did not simulated systems in vitro, because they were limited to in vivo experiments. It should be noted that the influence of growth and signaling factors is a key issue to understand adequately the morphogenesis and development of any tissue generated in vitro and in vivo.

In developmental Biology, Setty et al, modeled the Pancreas organogenesis [12]. His model is based on State Charts. Although, it considers genes activation and growth factors, they didn't define a language to model the systems components through which a non-computer expert could apply.

Although, previous works did a great contribution, their models did not consider a domain language that enable the comparisons between models. This work contributes in this area proposing a language that permit to model and its simulation using agents for visualization and prediction morphogenesis of biological tissues considering cascade genes, transcription factors and signaling methods like growth factors or morphogens.

This work is structured as follow. In the section 2 a metamodel developed will be detailed and biological concepts used in the language elaboration will be given. In the section 3 as case of study, is considered the morphogenesis of heart valve created in vitro. Also, will be showed the results obtained. Finally, in the section 4, our conclusions will be done.

2 Metamodel and language definition

A set of concepts were defined to model the expert domain. To indicate the words considers in the language they are written in italic font (see Fig. 1).

Cell is the main concept defined, they are the functional unit of life. A *cell* has a life cycle: born, reproduces and dies. According with environment setting, a cell can be of different types. For instance, stems cells, activate interstitial valve cells (aVIC), quiescent interstitial valve cell (qVIC), Ductal Pancreatic Cell, Pancreatic Beta Cell, Pancreatic Acinar, Endothelial Cell, Neuron, among thousands of others. During its life, some *Cells* can change its type. For example, a cell born as a stem cell but then grow up as activate interstitial valve cell. This phenomenon is known as differentiation. Each type of cells has a different behavior and properties. In the metamodel, it has been represented as concept *Cell Type* associated with the main concept *Cell*. This representation allows modeling that cell never change its essence but changes its behavior and characteristics. During its life cycle a cell can change its type, but keep on been a cell. Also, *cell* is considered an abstract concept which is specialized according with the specified *Cell Type.* A *Cell* could be in contact with another's forming aggregates. That is why, we modeled the concept *Contacts* to represent their attachment means, which are specific structures in its cellular membrane that also permits communication between them. The *aggregates* is composed of one or more kinds of *cells* and conform a *topology*. Every *topology* defines an identifiable characteristics spatial

pattern of specific *Type Tissues*. *Tissue* is also an abstract concept, whose behavior is implemented by a specific *Type Tissue.*

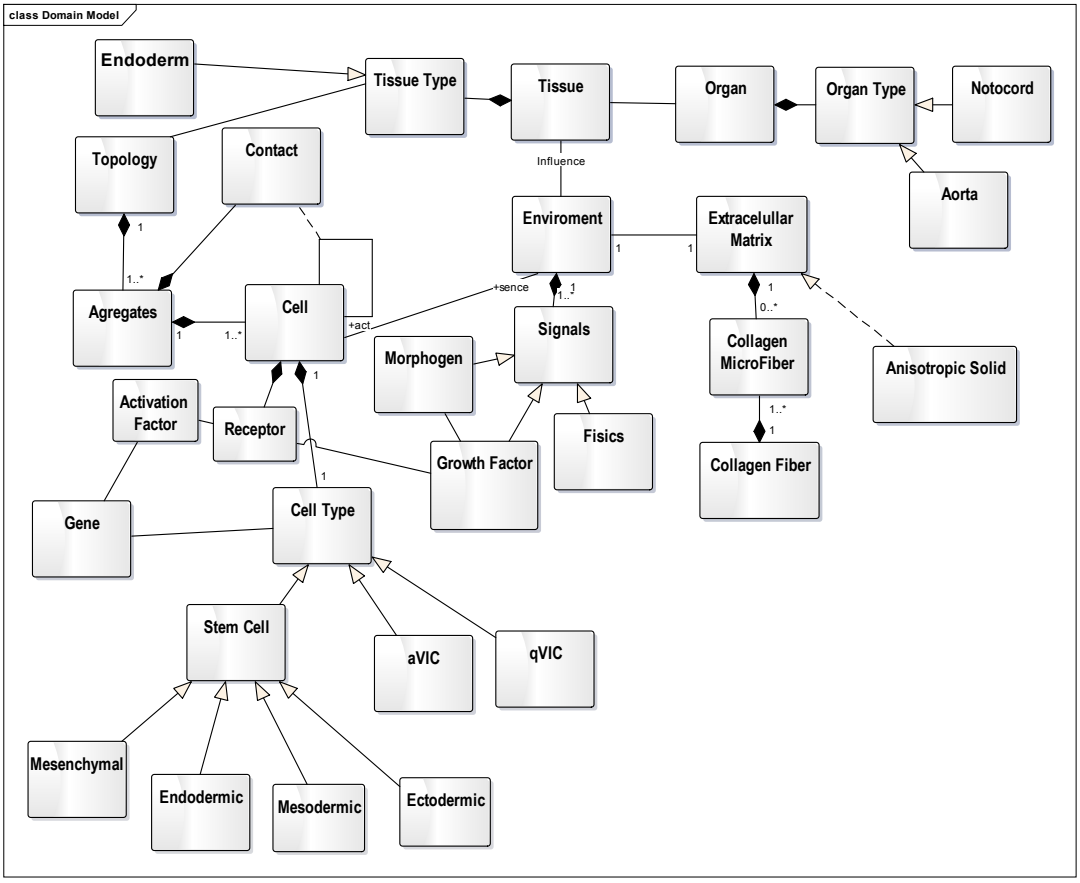

Fig. 1. Metamodel. The figure show the relashionship between language concepts

Depending of abstraction level, in some occasion is necessary to model the influence of set of cells, like tissues or organs on the environment. Because of that, we defined the *Tissue concept, a Tissue* set conform an *organ*. An *organ* could be conformed of many different *type tissues*. There are primitive organs, like *notochord*, which exist only during the embryo development. This kind of organs coordinate the organogenesis of others. Also, there are tissues that conform part of systems like the Aorta, that compound the cardiovascular system, and exist also after determinate embryo developing stages.

Other important concept is the *Environment.* The *Cell* sense and act in the environment and develop its social life. In their Environment *Cell*s are communicating with another using chemical *Signals* called *Growth Factor* and *Morphogen*s, but also *sense Physical Signals* like mechanical strain. On its membrane a *Cell* has specific receptors for *signals*. The concept *Receptor* represent the structures that permits to a

Cell receive signal of the environment. Some of these signal are *Growth Factors* [6]. This concept represent chemical molecules sent as messages by *Cell*s. When a *Cell* sense a number a *Growth Factors* above of threshold value, a sequence of reactions began and produce, as result, an *Activation Factor*. The *Activation Factor* concept, represent a protein necessary to transduce a *gene* by ARN polymerase enzyme . Later, the *gene* will be expressed and it could change the *Cell Type.* The *Morphogen* is a molecule, which could be or not a *Growth Factor*, that indicate to *Cell* their position on the *Environment* [5] Some of this concepts represent cell internal mechanism that will be useful when it is necessary to consider morphogenesis process explained which genes activation.

The *environment* is the cellular habitat that have to support fiscally cells and molecules. It can be composed of natural *extracellular matrix* (ECM) or a temporal material that is gradually degraded and replaced by the natural extracellular matrix. The *ECM* is composed of molecules that are produced by cells. A kind of molecule secreted by cells and deposited on ECM are *collagen microfibril*s that might be orientate by mechanical stress. *Collagen microfibrills* can self-assembly by joining each other and conform *collagen fiber*s that have strength. There are so many other substances, some of them act as signaling deposited on the environment. The ECM is considered an *anisotropic solid* because it is deformed with different values when on it is applied a mechanical stress.

3 Study Case: Heart Valve Morphogenesis, In vitro

To demonstrate the use of concepts defined in the language was considered the morphogenesis of heart valve in vitro.

A heart valve is composed of two or three resistant membranes called valves. The resistance of these valves is determined by the number and density of the crosslinking collagen fibers which are composed of collagen microfibrils. There a diverse cells population within heart valve, some of then are VICs (Valve Interstitial Cells), see **Fig 2**. There 3 types of Vic: pVic, aVic, qVic. These cells originate from an undifferentiated cell type called pVIC that are normally obtained from the bone marrow. This kind of cell can divide into more cells pVIC or differentiated to another type called aVIC (activated Valve Interstitial Cell) which seem to be a myofibroblast. The aVICs can produce high amount of collagen microfibrils. Also, they could differentiated to qVICs (quiescent Valve Interstitial Cell) similar to a fibrocyte and produce collagen microfibrils when do maintain functions [7].

The environment in which cells and collagen fibrils reside, is called intercellular space. In their natural environment, the intercellular space is an extracellular matrix construct by the same cells, but in in vitro experiments, an artificial environments that allow cell culture. It is used as a temporary support while the cells recreate its own extracellular matrix. Cells secreted substances, some of them are signals such as growth factors, other conform the ECM like collagen microfibrils. These microfibrils self-assemble and form stronger fibers when receiving mechanical signals of the me-

dium. The environment, also influence cells behavior, they are influenced by concentration of growth factors or directional signaling like chemotaxis molecules and mechanical stress and ECM deformation

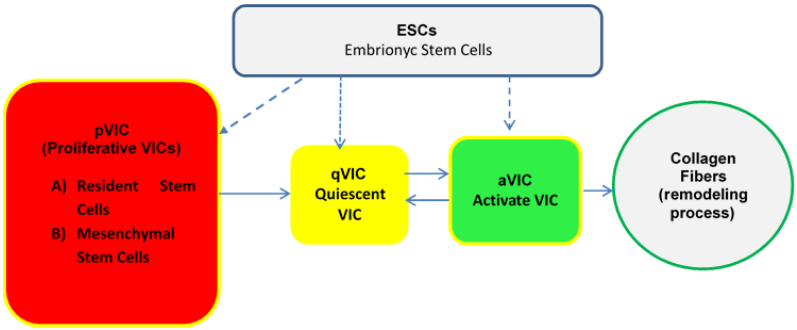

Fig. 2. Relation between cell types that compound the under study system

In this example, consider using mesenchymal stem cells (MSCs or pVICs) found in bone marrow. When a pVIC receive molecules of TGF-beta1 (Transforming Growth Factor Beta 1), it will reproduce and diferenciate to aVIC. Other factor involved in the system is FGF-2 (Fibroblast Growth Factor 2) that indicate to pVIC that have to reproduce and remain undifferentiated. Growth factors have the property of activating the cells only after reaching a threshold concentration below which its presence has no effect. The values of these thresholds are selected at the beginning of the simulation.

3.1 Definition of agents

As rule, to map language concept to an agent based model, we considered the Rusell and norvig definition: “agent is any entity that sense the environment and act on it” [10]. The *Cell* concept was implemented using an agent called Agent VIC. This agent has a counter, whose value determines its position in the cell cycle. A cell needs time to grow and differentiate. To specify your conduct, considered in its state corresponding *type cell*. It also has a variable that indicates its status and is linked to *Type Cell* concept implementation. 3 types are involved, Mesenchymal Stem Cell (pVIC) Valve Interstitial Cell (aVIC) and valve quiescence inactive cell (aVIC) were modeled. Thus the possibility of changing from one type to another by changing their behavior without losing their identity as flexible cell. The Type Cell class is an abstract class, derived by inheritance classes that implement the characteristics and behaviors of each cell type considered. Consequently, the differentiation process involves a change of status involved *Type Cell* specific instantiation.

There are molecules that act autonomously. This is the case of collagen microfibrils. These agents called Co and it can form a compound Collagen Fiber Agent that implements the behavior of the collagen fiber.

While growth factors influence the system dynamics agents are not considered as the metamodel not consider their mechanism of action, being process further easily

behavior is modeled by diffusion equations. They have a threshold for activating cellular actions.

The Vic Agents has an internal counter that is increment in every simulation steep. The purpose, is to indicate the cell position in its vital cicle. Every state transition has a constant value from which the cell could change. On aVic state, when the counter is greater than cReproduction (constant value equal to 30) the aVic agent can create two himself copy, one copy occupies its position, the next occupies an empty neighbor position. Besides, a constant value named *Dif-qVic = 25* is defined to indicate the number of steep simulation in which the Vic agent could pass from aVic state to qVic state (start the differentiation process from aVic to qVic). Furthermore, the transition from pVic to aVIC could be reached only if its internal counter in greater than *cDif-Avic= 20.*

aVIC agent state defines the agent behavior to simulate the stem cells, is called to be a mainly pVIC proliferative state, but the state can change to aVIC, as the value of the internal counter and the following rules that define their behavior.
The behavior of the agent in pVIC state, was modeled considering the effect of growth factors and physical space. If the cell has not space, it can't reproduce. Besides, if the Agent in pVic state receive an amount of TGF-Beta 1 above its threshold and if the internal counter is above cReproduction cycles, the reproduction start and to create 2 copies, and died, every copy start its internal counter at 0. This rule interprets the fact that a stem cell cannot be reproduce immediately, need to reach to maturity, and it will do if the signal are received (as TGF-beta1 and FGF-2 factor). If the FGF-2 is above its threshold remains its kind, receive or not TGF-beta1. If it receive one TGF-beta1 and its internal counter is above cDiff-avic cycles, then changes to qVic state. The AVIC state represents the behavior of myofibroblasts. These cells are responsible for creating microfibers that self-assembly to fibers. The collagen microfibrill behavior is interpreted by the fibrill agent Co. Consequently, the VIC Agent creates in each simulation steep one agent Co. Also, it remain its state when receive TGF-beta1 or differentiate to qVic if it comes in contact with neighboring cells (contact inhibition).

The qVic cell protract, trying to reach other qVic cell, when it happens, the qVic cells joint between them. It is very difficult to know precisely what information these cells share, one hypothesis is that among other things they share the value of deformation on its environment [7]. The cell does not directly senses the stress, because it has no sensors for that, it sense the strain, for which it have sensors. The strain is classified as low, average or high. The agent VIC, in qVic state, will link to another when there are in a distance less than D (D is a constant parameter set at the simulation beginning) and become to aVic state if the strain sensed is high.

An Agent Co, has a simple but crucial role in the behavior of the system. It has two possible states: routed and non-routed. When the VIC creates agent Co, it is located in the same position with random orientation. Next, it senses the direction of stress applied on the environment and will routed towards it. Besides, if an agent Co found another at a close distance, joins him at the tip to form longer segment, or on the side to form thicker segments.

The Simulation Environment is linked to Extracellular Matrix. First, we define the Extracellular Matrix (ECM), that is modeled as a square area of 100 x 100 micrometers, which it is divided in sectors 5x5 micrometers. Simulates the behavior of a solid anisotropic (when a stress is applied, it is strained nonlinearly with different values for different directions). At the beginning of each cycle, the environment provides the information request by the agents. At the end of every simulation step, the environment must operate applying the agent actions and updating its state.

3.2 Results

The model was implement on Netlogo and tested using many scenarios, because of paper space only is presented when the Stem cells are seeded with TFG-Beta1 and stress on Y direction of the environment. On Figures 3 (a, b, c, d) show recreated patterns that are similar to the observed in vitro and obtained by Liu et al [7] and Mulholand et al [9]. In Figure 3 (e) shows that it has reached the confluent state, while Figure 3 (f) indicates that all the collagen has been addressed following tension. The initial conditions of simulation: cells 10 Stress X = 0; Stress Y = 10kPa; FGF-2 = 0; FGF-2 Threshold 1.02 ng / ml; FGF-2 consumption = 1.02 ng / ml; FGF-2-rate = 0.2. TGF beta 1 = 0.97 ng / ml; TGF beta 1 threshold = 0.1 ng / ml; TGF-beta1 consumption = 0.1 ng / ml; TGF-beta1 rate = 0.75.

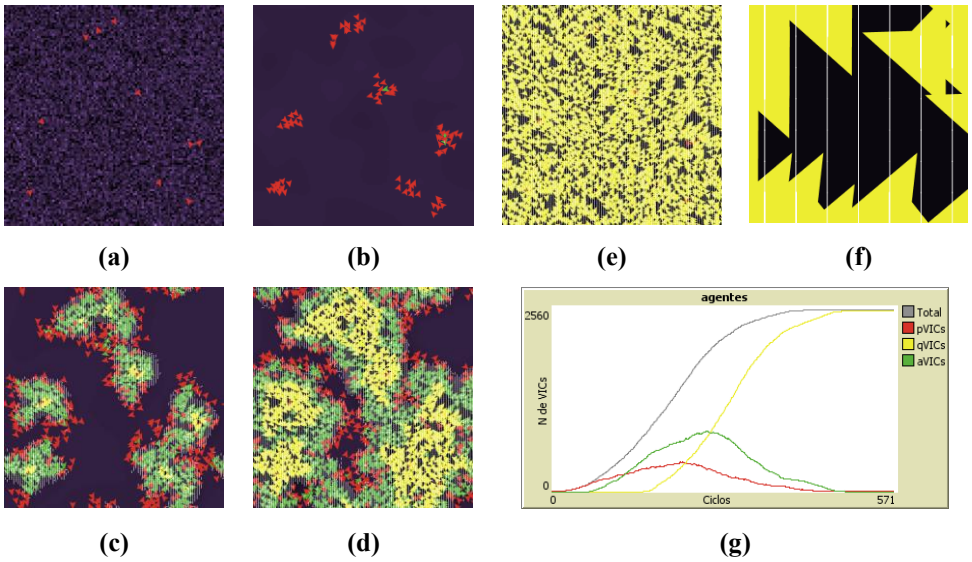

Figure 3 (a) initial state, (b) 50 simulation cycles. (c) 150 cycles of simulation; (d) 250 simulation cycles. (e) state of confluence, 600 simulation cycles. (g) Cell population vs simulation step.

4. Conclusions

The defined language represents the concepts domains. Through its implementation in Netlogo was possible to recreate the regenerative process of a heart valve in vitro. Agent-based modeling is a tool that simplifies the process and allows mapping from entities domain concepts simply. The rules that define the behavior of agents are suitable to recreate the dynamics of the process, considering that biological entities are not linear, the method modeled properly these systems, which generally are on the border of chaos and sensitive to their initial conditions. The model created and its simulation illustrates that it is possible to recreate emergent phenomena emphasizing the behavior of the system components and their interaction. The model allows to predict situations that happen in experiments in vitro and it is useful as tool for testing hypotheses and theories that can be experimentally checked. The language created, hide the implementation details and execution of a simulation.

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